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Laboratory Culture of Triploid Grass Carp

Alberta
ENVIRONMENTAL CENTRE

Laboratory Culture of Triploid Grass Carp

by

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SUMMARY

In 1987 the Government of Alberta embarked upon a major program aimed at the use of grass carp (Ctenopharyngodon idella) as agents in the control of aquatic weeds in irrigation canals. A significant obstacle to the program was Alberta's low surface water temperatures, which are generally unsuitable for spawning and the development of fry. Hence, staff at the Alberta Environmental Centre had to develop techniques for the laboratory culture and transport of carp. Subjects that are covered in this report include: i) source, transport and receipt of carp, ii) physico-chemical maintenance conditions, iii) feed, iv) growth, v) spinal deformities, and vi) disease diagnosis. Two methods (Coulter Channelyzer, Chromosome Analysis) were also developed and implemented for the determination of ploidy in carp.

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1. INTRODUCTION

1.1 Background

The grass carp (Ctenopharyngodon idella) is a large herbivorous member of the minnow family (Cyprinidae). It was originally found throughout much of eastern China, but has been recently introduced into more than 50 countries. The grass carp has appeal for two reasons: i) it is important in fisheries in many countries, both commercial and domestic, because of its size, production, and palatability and ii) it is a highly effective agent in the control of aquatic weeds. Under favourable temperature conditions (25-28°C), the grass carp can consume 2-3 times its body weight in vegetation per day. Sexually sterile fish, containing a triploid number of chromosomes, can also be produced, thereby eliminating the possibility of reproduction in the wild.

Whether or not the use of grass carp finds favour with fisheries' managers depends on their approach to perceived and actual problems associated with the introduction of exotic species. Petridis (1990) noted that grass carp, through moderate weed consumption, produced better conditions for exploitation of benthic organisms by benthophagous fish. The weed consumption also increased the amount of open water area, thereby increasing the availability of zooplankton to planktivorous fish. On the other hand, grass carp may be a threat to valuable fish and wildlife habitat (Pflieger, 1975; Fedorenko and Fraser, 1978). Under extreme conditions, the roots of plants growing

along shorelines may be eaten and the vegetation dragged into the water (American Fisheries Society, 1987).

Despite these potential problems, the major alternative for weed control, the use of chemical herbicides, probably poses more problems to the aquatic environment than does the use of grass carp. Alberta has over 12,000 km of irrigation canals, many of which are treated each year with acrolein. The usual application dose of acrolein is 5-8 mg/L, well above the 96-h LC_{50} of <0.1 mg/L (Alexander et al., 1985). Similarly, agricultural dugouts used for livestock water and seasonal trout farming often need to be chemically treated, potentially harming water users. It therefore seems that the use of grass carp to control weeds will expand.

Grass carp have never been intentionally released into Canadian waters. In fact, there is only one record of grass carp occurring in Canada, a single male caught in Lake Erie (Crossman et al., 1987). The fish weighed 5.3 kg, with a fork length of 70 cm, and had likely migrated from a tributary on the American side of the lake.

1.2 Purpose of Program

In 1987 the Government of Alberta embarked upon a major program aimed at the use of grass carp as agents in the control of aquatic weeds in irrigation canals. A significant obstacle to the program was Alberta's low surface water temperatures. Spawning normally takes place at 27-29°C, and fry require water of 20°C or more to grow well

(Wheeler, 1975). Since such conditions are rarely found for long in Alberta, indoor culture and maintenance facilities had to be developed.

The purpose of this report is twofold: 1) to describe indoor culture methods used for grass carp, and 2) to describe two methods for determining chromosome number in reportedly triploid fish. Because some of the methods used to rear the grass carp have not been previously described, detailed experimental protocols are appended to this report.

2. SOURCE, TRANSPORT AND RECEIPT

Approximately 5,000 6-day-old larvae were airshipped from the Lee County Hyacinth Control District (Fort Myers, Florida) to Edmonton and then trucked to Vegreville (Table 1). Although transit time was 18 h, mortality was low, <3%. Upon arrival at the laboratory, warm (22°C) dechlorinated water was added over a 2-4 h period to the shipment bag. The larvae were then transferred to two 125-L aquaria containing water of 22°C (pH 8.45; dissolved oxygen 8.3 mg/L; conductivity 240 µS/cm).

Table 1. Source and transport of larval grass carp.

Source:	Lee County Hyacinth Control District, Fort Myers, Florida
Number and Age of Larvae:	Approximately 5,000, 6 days old
Size of Larvae:	Average total length, 7 mm Average wet weight, 1.5 mg
Mortality in Transit:	Approximately 3%
Transit Time:	18 h
Shipment Container:	Polyethylene bag, 8 L water
Water Quality:	Temperature, 26°C (source) 18°C (destination)
	pH, not known (source) 8.3 (destination)
	Total NH_3, not known (source) 0.22 mg/L (destination)

3. PHYSICO-CHEMICAL CONDITIONS

All fish were maintained under quarantine conditions throughout the study. When the fish were small (<15 cm in fork length, age 6-30 days) they were held in aquaria, then moved to fry troughs at age 31-60 days (fork length 15-25 cm). Circular 800-L tanks were used for maintenance beyond 60 days (>25 cm in length). Water temperatures were maintained at 20-25°C throughout the study. Dissolved oxygen was maintained at >60% saturation and conductivity at approximately 240 µS/cm.

4. FEED

The feeding regime described here is based on standard procedures used at the Alberta Environmental Centre (AEC), plus a series of experiments described in Appendices 1-12.

Since larval grass carp are naturally planktivorous, they were first presented with live larval brine shrimp (Figure 1). A commercial larval fish food (AP-100, Zeigler Brothers, Gardner, Pennsylvania) was introduced at age 16 days to accustom the fish to a non-animal material. Frozen adult brine shrimp were first presented at age 25 days, and the larval brine shrimp were deleted from the diet.

Commercially available trout feeds formed the major part of the diet beyond day 45 (Figure 1). The size of pellet increased gradually. Floating feeds kept the tanks cleaner than sinking feeds. Alfalfa pellets and rabbit pellets were also presented to increase the quantity of carbohydrates and fibre in the diet. The final food item, catfish feed, was introduced at age 283 days. This product, high in vitamin C and other micronutrients, reduced the number of spinal deformities in the culture population (see Section 6, Spinal Deformities).

5. GROWTH

Changes in mean fork length followed a linear path throughout the study (Figure 2). At 420 days, the mean length of the carp was

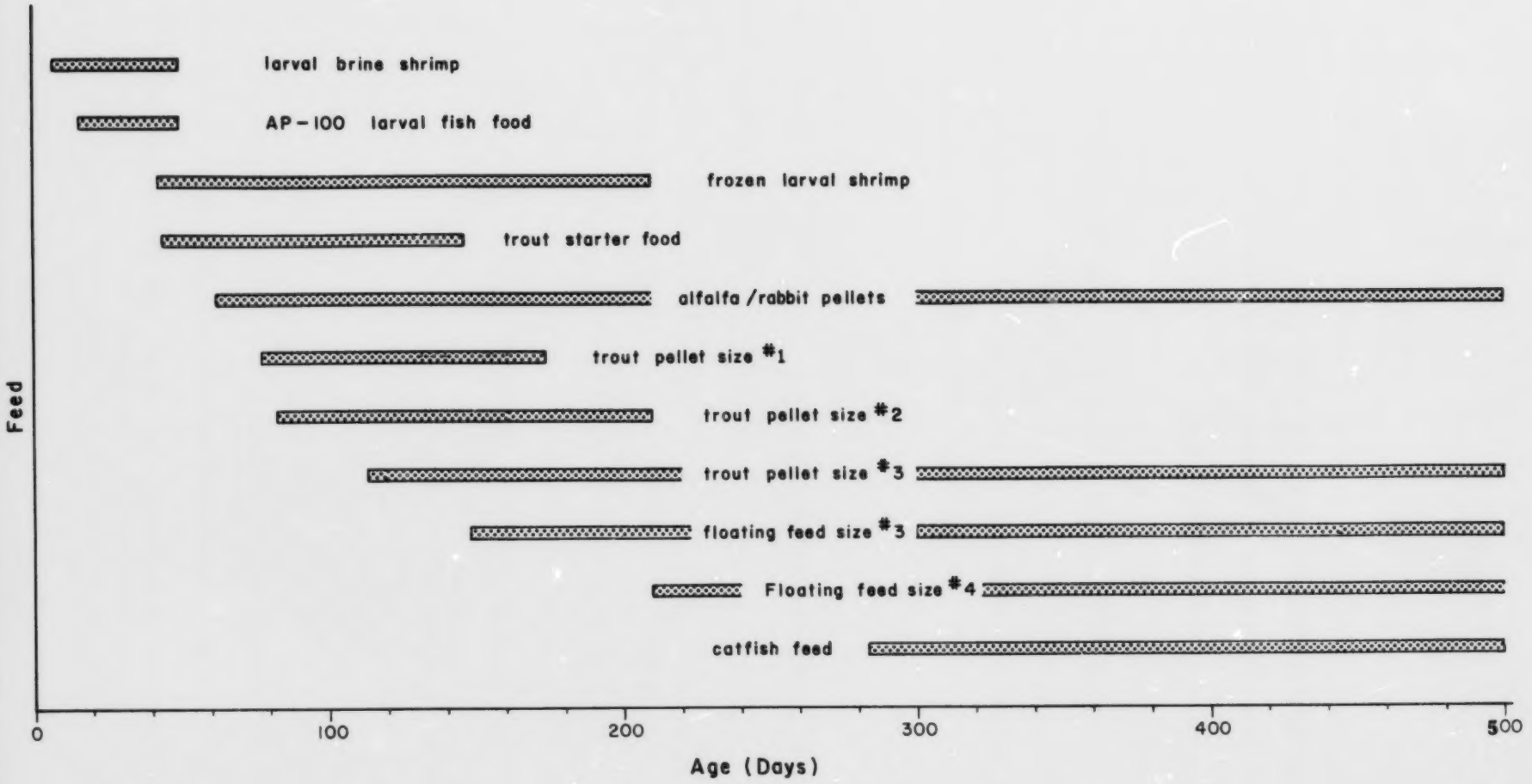

Figure 1. Feed for grass carp at different ages.

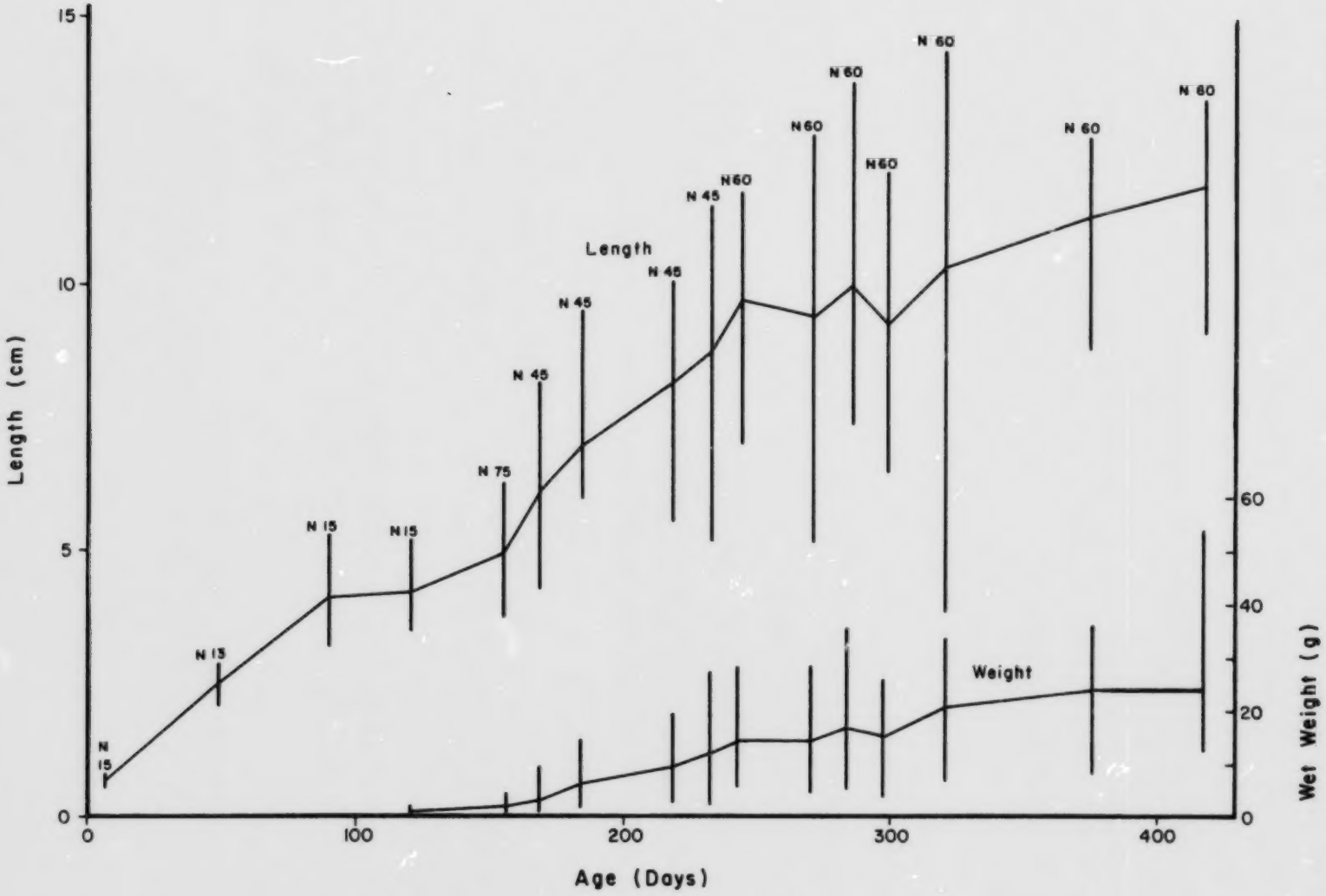

Figure 2. Changes in fork length and wet weight of laboratory - reared grass carp.

Vertical Bar: range

N: sample size

approximately 12 cm, with a range of 9 to 13.5 cm. Mean wet weight was <1 g for more than 120 days at 19°C, but then increased to 24 g by day 420 (Figure 2) after increasing the water temperature to 25°C. The heaviest fish in the population weighed 54 g at day 420.

6. SPINAL DEFORMITIES

Only a few (<0.1%) larval grass carp exhibited scoliosis during the first part of the maintenance cycle (day 7 up to day 100). The frequency of scoliosis did, however, increase to the point where 2.8% of the population suffered from it by day 223 (Table 2). The condition was generally severe, limiting the ability of fish to swim normally. The introduction of catfish feed, with its high vitamin C content, reduced the frequency of the problem to near 0% by day 313.

Table 2. Frequency of scoliosis before and after the introduction of catfish feed to the diet of grass carp.

Day	Number of Fish	Number of Fish With Scoliosis	Percentage of Fish With Scoliosis
223	4222	120	2.84
253	2899	51	1.76
283*	2799	2	0.30
313	2869	0	0
343	2191	20	1.10

*Introduction of catfish feed at day 268.

Another condition, blunting of the nose and mouth area, developed in most fish held over the long term. The frequency of this condition was close to 100% in fish held more than one year in the laboratory. Although the condition apparently developed when fish collided with the side of tanks, the movements and feeding behaviour of the carp remained unchanged throughout the length of the study.

7. DETERMINATION OF PLOIDY

Normal grass carp cells contain a complement of 48 chromosomes (diploid number). To induce triploidy, pressure shock is applied to freshly fertilized oocytes suppressing anaphase II, resulting in diploid female pro nuclei (48 chromosomes). When these oocytes fuse with haploid sperm (24 chromosomes), triploid embryos (72 chromosomes) are produced. These individuals have retarded gonad development. Although females may produce occasional oocytes, they are considered functionally sterile. Spermatozoa produced by the males bear an abnormal chromosome number (aneuploid) and, as a result, any gametes produced would also be aneuploid and likely not viable.

The induction of triploidy in fish is rarely 100% successful; therefore, screening techniques are required to identify triploid individuals before releasing them into the environment. Two procedures were implemented at AEC to screen for triploid

individuals. They are the Coulter Channelyzer and Chromosome Analysis methods.

7.1 Sampling and Preparation of Blood

Each fish, weighing >2-3 g, was anaesthetized with tricaine methanesulfonate (MS 222) in water. Blood was taken from the caudal vein near the caudal peduncle using a sterile heparinized syringe and 26 gauge needle. For erythrocyte volume assessment using the Coulter Channelyzer, 1 µL of blood was delivered into an Accuvette containing 20 mL of Isoton II and 2 drops of Zap-O-Globin II, using a micropipettor with disposable tips. The sample was analyzed for ploidy within 30 min of collection. For chromosome analysis, 2-3 drops of blood were added to 5 mL of culture medium.

7.2 Coulter Channelyzer Method

Triploid erythrocyte nuclei, with their extra set of chromosomes, are approximately 50% larger than diploid nuclei. This permits the use of Coulter instrumentation to measure nuclear volume differences.

7.2.1 Coulter Channelyzer Instrumentation and Calibration

A Coulter ZBI with a 70 µm orifice aperture tube and a Coulter channelyzer Model 256 were used in the analysis. The ZBI was precalibrated with latex microspheres using the half-count method recommended by the manufacturer. Instrument settings were optimized so that all cell volumes were fully displayed on the monitors.

Final calibration was achieved by determining the peak channel in which 8.6 fL latex spheres were recovered. The resulting calibration constant (K_c) of 23.070 was obtained. The K_c remained fixed throughout the study. To check calibration, peak channels were determined using a mixture of 4.32, 8.60 and 16.21 fL spheres. The 16.21 fL spheres appeared as triploid nuclei and the 8.60 fL spheres as diploid nuclei. In addition, this mixture was reassessed after every 10 samples to ensure that the system remained in calibration. The calibration particles were consistently recovered in their corresponding channels (Table 3).

7.2.2 Coulter Channelyzer Analysis

Before sample analysis, a background count was conducted using the diluent. As blind control, samples consisting of erythrocytes from fathead minnow (Pimephales promelas) were analyzed. These minnows were used because their red blood cell nuclear volume closely resembles that of diploid grass carp.

Table 3. Recovery of latex spheres in different channels.

Particle Size	Size (fL)		Channel Number	
	Mean	Range (2SD)	Mean	Range (2SD)
4.32	4.44	4.28-4.60	29	28-30
8.60	8.54	8.34-8.70	54	53-55
16.21	15.91	15.87-16.07	99	98-100

One thousand nuclei in the peak channel were counted for each sample. The histograms were printed and ploidy determined by the use of the criteria listed in Table 4. Fish that tested in the range of 11.7-12.4 fL were considered diploid.

Table 4. Criteria used to assess ploidy in grass carp.

Ploidy	Volume Range (fL)
Diploid	8.5-11.6
Triploid	12.5-18.6
Tetraploid	>18.6

Source: Wattendorf (1986).

7.3 Coulter Channelyzer Results

Erythrocyte nuclear volume was determined for 1626 fish. Of this, 1593 (98.0%) were triploid, 27 (1.7%) were diploid, 4 (0.2%) were triploid/tetraploid mosaic, 1 (<0.1%) was diploid/triploid mosaic, and 1 (<0.1%) was a tetraploid. The volume of the nucleus of erythrocytes from triploid fish averaged 14.30 fL compared to 9.48 fL for diploids (Table 5).

7.4 Chromosome Analysis Procedures

Chromosome spreads were prepared from leukocytes using the micro technique adapted from human studies (Moorehead et al., 1960). The culture medium consisted of 70% Medium 199, 30% fetal calf serum, 0.5% pen/strep, 1% heparin, 2-4% Phytohemagglutinin M. After the

addition of the blood sample, the culture was incubated in the dark at 22-24°C for 120-144 h. Two h prior to harvest, 0.1 mL of Colcemid was added to arrest mitosis at metaphase.

Harvesting of the culture and preparation of the slides was performed using a modified Legendre's (1975) method. To ensure complete cell membrane disintegration, the hypotonic solution (0.075 M KCl) was left on the cells for an additional 15 min. The slides were stained with 10% Giemsa solution (pH 6.8-7.2) for 5 min. Five counts of each sample were made under 100x oil magnification.

Table 5. Comparisons of nuclear volume of erythrocytes of triploid and diploid grass carp.

Ploidy	Sample Size	Size (fL)		Channel Number	
		Mean	Range (2SD)	Mean	Range (2SD)
Triploid	1593	14.30	13.98-14.62	90	88-92
Diploid	27	9.48	9.16-9.80	60	58-62

7.4.1 Chromosome Analysis Results

The total number of chromosome analyses performed was 176, of which 65 (37%) yielded sufficient metaphases for evaluation. The ploidy determination by chromosome analysis was consistent with the Coulter method in all but two cases. In these, the channelyzer revealed a mosaic whereas only one stem line was demonstrated by chromosome analysis.

8. HEALTH CHECKS AND DISEASE DIAGNOSIS

Routine health checks were completed monthly on 10 carp. The fish were anaesthetized, then examined for gross lesions and preserved in Bouin's solution. All major tissues including skin, eye, gill, buccal cavity, liver, kidney, pancreas, spleen, GI tract and heart were examined for potential lesions. No bacterial, viral or parasitic agent, significant to the health of carp, were associated with laboratory-held fish.

9. DISCUSSION

Grass carp can be easily reared under laboratory conditions. During the 420-day course of this study, mortality was extremely low, and there was no indication of disease induced by parasitic, bacterial or viral agents. Mortality during transport from the supplier in Florida and later to the client in Lethbridge was essentially nil.

The major drawback of the laboratory culture of grass carp centers around cost. The processes of feeding, tank cleaning, determination of growth, and maintenance of water quality requires at least one person/yr. The provision of food, chlorine bleach for disinfection of effluent from the holding tanks, filters, nets and consumables is also expensive, exceeding $5000/yr.

Another major cost is that of treated water. Because the carp must be maintained on a flow-through, quarantine system, the consumption of water is large. Peak flows of 60 L/min were required for maintenance when the grass carp population was at its heaviest. This resulted in an expenditure for water of approximately $155/day. Such costs are not encountered when carp are raised in dugouts or ponds.

The only other potential drawback is the relatively slow growth rate of laboratory-reared carp. In our tanks, the average weight of fish was 35 g after one year growth. Under natural outdoor conditions, carp may weigh more than 1 kg after one year (Shireman and Smith, 1983). Small carp, once released to canals, are likely to consume fewer weeds than large fish, and are more susceptible to predation by birds and larger fish. The reason for the slow rate of growth of laboratory-reared fish may have been our inability to provide feed on a 24-h basis. Being a herbivore, grass carp eat slowly over extended periods. It was not possible to continuously feed the fish in our laboratories because the maintenance water would deteriorate to the point where it would be unsuitable for the carp.

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APPENDIX 1

ASSESSMENT OF COMMERCIAL FEEDSTUFFS FOR GROWTH OF GRASS CARP FRY REARED IN TANKS

1. INTRODUCTION

Although grass carp growth is best achieved by feeding natural vegetation, this is impractical for a long-term, high-density tank culture system. Grass carp fry can utilize animal matter (e.g. trout feed; brine shrimp) but they also require some vegetable matter and fibre. Hence, the objective of this study was to evaluate the growth of grass carp fry fed commercially available feedstuffs.

2. STUDY DESIGN

Three combinations of commercial feedstuffs were evaluated:

a. 50% trout feed, 45% brine shrimp, 5% alfalfa;

b. 70% trout feed, 25% brine shrimp, 5% alfalfa; and

c. 95% trout feed, 0% brine shrimp, 5% alfalfa.

Details of the method are listed in the experimental protocol (Appendix 7).

3. RESULTS

Analysis of variance did not detect differences ($p>0.05$) among the three feeding regimes based on final weight, final length or the

percent change in each parameter (Table 1). The fish fed commercial feed mixture b gained approximately 7% more body weight in the 14-day study than when fed on other mixtures. Hence, feeding regime b was implemented as the standard feed combination for initial feeding of grass carp fry once placed into maintenance tanks. Alfalfa pellets, crumbled to the same size as the trout feed crumbles, were fed as 5% of the daily ration to all fish as a source of fibre and vegetable matter.

Table 1. Mean (±SD) weight (g) and fork length (cm) of grass carp fry fed commercial feedstuffs.

Growth Parameter	Feed Treatment Group*		
	a	b	c
Initial weight	0.79 (0.33)	0.92 (0.28)	1.00 (0.32)
Final weight	0.88 (0.28)	1.08 (0.36)	1.10 (0.32)
% Weight gain	10.8 (35.1)	17.7 (38.8)	10.5 (32.6)
Initial length	3.6 (0.5)	3.7 (0.5)	3.8 (0.4)
Final length	3.8 (0.5)	4.0 (0.5)	4.0 (0.4)
% Length gain	5.2 (13.8)	8.1 (12.2)	4.9 (11.5)

a = 50% trout feed, 45% brine shrimp, 5% alfalfa; b = 70% trout feed, 25% brine shrimp, 5% alfalfa; c = 95% trout feed, 0% brine shrimp, 5% alfalfa.
*Treatment means are not different.

APPENDIX 2

EFFECT OF WATER TEMPERATURE ON GROWTH OF TANK-REARED GRASS CARP

1. INTRODUCTION

Feed intake and growth of grass carp are directly related to water temperature. Improper feeding rates in relation to water temperature could affect growth and lead to nutrient deficiencies. Overfeeding can contribute to husbandry problems because of reduced water quality resulting from waste feed and metabolic products in the water.

The objective of this study was to evaluate the effect of water temperature on the expected growth of grass carp reared in tanks.

2. STUDY DESIGN

a. Water temperatures were 20, 25 and 30°C.

b. Grass carp fry averaging 1.1 to 1.4 g body weight were fed at the rate of 10% body weight per day.

c. Treatment groups comprised of 15 grass carp were arranged in a completely randomized design.

d. The standard feeding regime of 70% trout feed, 25% brine shrimp and 5% alfalfa was fed for 14 days.

Details of the method are listed in the experimental protocol (Appendix 8).

3. RESULTS

a. Covariance analysis of final body weights, using mean initial weight per group, indicated the differences among the treatment groups were due to variation in initial weights and not due to treatment effects (Table 1).

b. Maximum growth (23.9% gain in mean body weight) with fish held at 25°C was not different ($p>0.05$) from the other treatment groups.

c. Increasing the water temperature from 25 to 30°C had no additional effect on weight gain.

d. Fish held at 20°C gained only about one-half (12.6%) of the mean body weight increase of the warm water groups.

e. A water temperature of 25°C was selected as the optimum for culturing grass carp in tanks.

Table 1. Mean (±SD) weight (g) and fork length (cm) of grass carp fed commercial feedstuffs at three water temperatures.

Growth Parameter	Water Temperature (°C)[a]		
	20	25	30
Initial weight	1.11 (0.30)	1.44 (0.25)	1.10 (0.32)
Final weight	1.25 (0.33)	1.78 (0.30)	1.36 (0.36)
% Weight gain	12.7 (29.3)	23.9 (20.5)	23.5 (32.5)
Initial length	4.2 (0.4)	4.5 (0.3)	4.1 (0.4)
Final length	4.3 (0.4)	4.9 (0.3)	4.6 (0.4)
% Length gain	2.7 (9.0)	10.8 (7.2)	11.9 (9.6)

[a]Treatment means are not different ($p>0.05$).

APPENDIX 3

EFFECT OF DIFFERENT FEEDING RATES OF COMMERCIAL FEEDSTUFFS ON GROWTH OF TANK-REARED GRASS CARP

1. INTRODUCTION

Grass carp consume more feed as water temperature increases; however, overfeeding of tank-reared fish can result in water quality problems when fish are held at high loading densities.

The objective of this pilot study was to evaluate the effect of different feeding rates of commercial feedstuffs on growth of grass carp reared in tanks at 20°C and 25°C.

2. STUDY DESIGN

a. Grass carp averaging ~2 g body weight were held at 20 and 25°C in 37-L aquaria.

b. Fish were fed at 8, 10 or 12% body weight per day.

c. The standard feeding regime of 70% trout feed, 25% brine shrimp and 5% alfalfa was fed for 14 days.

d. Fifteen grass carp were assigned to each treatment group.

e. The experimental design was a 2 x 3 factorial with two temperatures and three feeding regimes.

Details of the method are listed in the experimental protocol (Appendix 9).

3. RESULTS

a. Overall weight gain of fish held at 20°C was ~42% of initial body weight while fish in 25°C water gained ~52% of body weight (Table 1). These means were significantly different ($p<0.05$), again indicating the influence of water temperature on grass carp growth.

b. The overall effect of feed rate on mean percent weight gain was not different ($p>0.05$); however, the temperature x feed interaction was significant ($p<0.05$), due largely to the combination of an 8% feeding rate at 25°C (Table 1).

c. Although not significant ($p>0.05$), feeding rates of 8 and 10% produced about 10% greater increase in body weight than did the 12% rate (Table 1), indicating that there was no benefit in feeding 12% body weight per day at either temperature.

d. The largest weight gain (61.7% of initial body weight) occurred with fish held at 25°C and fed 8% of body weight per day.

e. Statistical partitioning of feeding rate x temperature treatment combinations indicated that percent weight gains ranging from 50 to 60% produced by feeding 8% and 10% body weight at 25°C, and 10% body weight at 20°C were comparable.

f. No differences ($p>0.05$) among treatment means of fork length data were detected (Table 2).

g. Larger amounts of feed were not consumed but contributed to water quality and maintenance problems.

h. A rate of 8% body weight was adopted as the daily feeding routine for grass carp of this size held at either 20 or 25°C.

Table 1. Mean weight (±SD) of grass carp held at 20 and 25°C and fed at 8, 10, and 12% body weight per day.

Growth Parameter	Water Temperature °C	Feeding Rate (% Bd. wt./day) 8	10	12	$\bar{x}$
Initial weight (g)	20	1.98 (0.27)	1.99 (0.29)	2.08 (0.17)	2.02 (0.24)
	25	1.96 (0.31)	1.89 (0.23)	1.96 (0.27)	1.94 (0.27)
	$\bar{x}$	1.97 (0.28)	1.94 (0.26)	2.02 (0.23)	
Final weight (g)	20	2.72 (0.40)[a]	2.97 (0.46)	2.89(0.48)	2.87 (0.45)
	25	3.17 (0.23)[a]	2.93 (0.51)	2.81 (0.46)	2.97 (0.44)
	$\bar{x}$	2.95 (0.39)	2.95 (0.48)	2.85 (0.47)	
Body weight gain (%)	20	37.4 (20.2)	49.3 (23.3)	39.2 (23.1)	42.1 (22.1)*
	25	61.9 (11.9)	55.1 (27.0)	43.4 (23.7)	53.4 (22.1)*
	$\bar{x}$	50.1 (20.4)	52.1 (24.9)	41.3 (23.1)	

[a]N = 14 after 14 days.
*Means are different at $p<0.05$. All other means are not different at $p<0.05$.

Table 2. Mean fork length (±SD) of grass carp held at 20 and 25°C and fed at 8, 10 and 12% body weight per day.

Growth Parameter	Water Temperature °C	Feeding rate (% Bd. wt./day)[a]			
		8	10	12	x̄
Initial length (cm)	20	5.1 (0.3)	5.1 (0.3)	5.2 (0.2)	5.1 (0.3)
	25	5.1 (0.3)	5.0 (0.3)	5.0 (0.2)	5.0 (0.3)
	x̄	5.1 (0.3)	5.0 (0.3)	5.1 (0.2)	
Final length (cm)	20	5.6 (0.3)	5.8 (0.3)	5.7 (0.4)	5.7 (0.3)
	25	6.0 (0.2)	5.7 (0.3)	5.8 (0.3)	5.8 (0.3)
	x̄	5.8 (0.3)	5.8 (0.3)	5.8 (0.3)	

[a]N = 14 after 14 days.
Note: Differences in mean fork length gains among treatments are not significant ($P>0.05$).

APPENDIX 4

FEEDING COMMERCIAL RABBIT FEED AS A SOURCE OF PLANT MATTER FOR TANK-REARED GRASS CARP

1. INTRODUCTION

Grass carp cannot digest fibre; however, fibre aids in digestion of animal feed. Trout feed used for feeding grass carp fry is mainly animal material because trout cannot digest vegetation. Rabbit pellets are about 14% crude fibre, are harder than pelleted alfalfa and are fortified with essential vitamins; consequently pelleted rabbit feed was evaluated as a replacement for alfalfa pellets in the feeding regime.

2. STUDY DESIGN

a. Treatment groups were 5, 10 and 15% pelleted rabbit feed combined with size no. 3 trout feed crumbles.

b. Each treatment group was comprised of two replicates of 10 fish arranged in a randomized complete block design.

c. Rabbit feed pellets were crushed and screened to the size of the no. 3 trout feed crumbles.

d. Grass carp ranged from 2.5 to 3.0 g body weight.

e. Fish were held at 25°C and fed at the rate of 8% body weight per day for 14 days.

Details of the methods are listed in the experiment protocol (Appendix 10).

3. RESULTS

a. Maximum growth in 14 days was obtained with fish fed 90% trout feed and 10% rabbit pellets (42.8% gain in weight) (Table 1).

b. Differences in mean weight gain among treatments were not significant ($p>0.05$). A feeding regime of 90% trout feed and 10% rabbit feed was established as a general procedure.

c. Replacement of alfalfa pellets with rabbit feed pellets reduced the amount of waste feedstuffs in the maintenance tanks.

d. Based on the above-noted results feeding of brine shrimp to grass carp >3 g was discontinued.

Table 1. Mean weight (±SD) of grass carp fed rabbit pellets as a source of plant material.

Growth Parameter	Trout feed:rabbit feed ratio (%:%)[a]		
	95:5	90:10	85:15
Initial weight	2.79 (0.16)	2.68 (0.16)	2.79 (0.16)
Final weight	3.65 (0.52)	3.83 (0.50)	3.81 (0.57)
% Weight gain	31.2 (19.0)	42.8 (19.2)	36.5 (20.4)

[a]Treatment means are not different ($p>0.05$).

APPENDIX 5

EFFECT OF FEED PARTICLE SIZE AND PRESENTATION OF COMMERCIAL FEEDSTUFFS ON GROWTH OF TANK-REARED GRASS CARP

1. INTRODUCTION

Underfeeding of grass carp and other species of fish, producing poor growth and nutrient deficiencies, can result because an improper feed particle size is fed. Particle size of feed should be directly related to fish size. Also, grass carp prefer feeding on matter suspended in the water column over bottom feeding.

The objective of this pilot study was to evaluate the effect of feed size and manner of presentation of commercial feedstuffs on growth of grass carp reared in tanks at 25°C.

2. STUDY DESIGN

a. No. 3 fish feed crumbles (3 mm) were fed to carp weighing 2.3-4.5 g body weight and no. 4 feed (4 mm) was used with fish weighing 4.6-11.4 g. These crumbled feeds sank.

b. No. 4 floating pellets were crushed and screened to the size of a no. 4 sinking trout crumble.

c. Treatment groups of fish weighing ~3.5 g (N=9) and ~6.5 g (N=5) were each fed no. 3 sinking, no. 4 sinking or a floating trout feed sized down to a no. 4 sinking crumble.

d. Daily feeding rates of 8.0% (no. 3), 9.6% (no. 4) and 10.4% (no. 4 floating) of body weight were used because the crude protein content of the three feeds was different. This maintained a constant daily intake of crude protein for each feed group.

e. Daily feed was 90% trout feed and 10% rabbit feed.

Details of the method are listed in the experimental protocol (Appendix 11).

3. RESULTS

a. The smaller fish (3.5 g) fed no. 3 sinking feed for 14 days gained 13.2% body weight and only 4.8% when fed no. 4 sinking feed; however, feeding a no. 4 floating feed produced a 16.8% increase in body weight (Table 1).

b. The no. 4 sinking crumbles were too large compared to no. 3 for proper utilization by 3.5 g grass carp. Growth of 3.5 g fish fed the no. 4 floating feed was comparable to fish fed the smaller no. 3 sinking crumbles.

c. The 6.5 g fish increased body weight by 27.5% when fed the no. 4 floating feed but only by 11.9% when fed the no. 4 sinking feed.

d. The effect of feed size on weight gain was not significant ($p>0.05$) due to the low sample sizes and high within-group variation.

e. Particle size of feeds and weight gain were related to fish size, and suspending the feed by using floating feed increased percent weight gain with both fish size groups.

f. Floating fish feed of the appropriate particle size was introduced into grass carp feeding regimes as fish attained the proper body weight.

Table 1. Mean (±SD) weights and percent weight gain of grass carp fed sinking and floating trout feed pellets.

Growth Parameter	Fish Size (g)[a]	Feed Group[b]		
		No. 3 sinking	No. 4 sinking	No. 4 floating
Initial weight	3.5	3.50 (0.35)	3.52 (0.37)	3.55 (0.38)
	6.5	6.65 (0.35)	6.61 (0.25)	6.71 (0.36)
	$\bar{x}$	4.62 (1.60)	4.62 (1.57)	4.68 (1.61)
Final weight	3.5	3.96 (0.41)	3.69 (0.62)[c]	4.15 (0.94)
	6.5	7.62 (0.85)	7.39 (0.79)	8.56 (1.28)
	$\bar{x}$	5.27 (1.91)	5.11 (1.99)	5.72 (2.42)
% Weight gain	3.5	13.2 (11.8)	4.8 (17.5)	16.8 (26.4)
	6.5	14.6 (12.7)	11.9 (11.9)	27.5 (19.0)
	$\bar{x}$	13.7 (11.7)	7.5 (15.5)	20.7 (23.8)

[a]N = 9 in 3.5 g fish size groups; N = 5 in 6.5 g fish size groups.
[b]Treatment means are not different ($p>0.05$).
[c]N = 8 after 14 days.

APPENDIX 6

GROWTH AND SURVIVAL OF GRASS CARP EXPOSED TO LOW WATER TEMPERATURES

1. INTRODUCTION

Although 25°C is the optimal water temperature for grass carp growth (Stickney, 1986), the water in southern Alberta dugouts when fish are released is much colder. The grass carp is tolerant to extremes in environmental conditions (Shireman and Smith, 1983); however, the tolerance of grass carp reared at the Centre to low water temperature needed evaluation before fish were released into cold water in southern Alberta. Also, the use of low water temperatures to retard grass carp growth without inducing nutrient deficiencies needed study.

The objective of this study was to evaluate the tolerance of grass carp reared in tanks at 5, 10, 15 and 20°C.

2. STUDY DESIGN

a. Test fish were fed 4 or 8% of body weight per day for 14 days.

b. Five grass carp weighing ~8 g were randomly assigned to each of the 8 treatment groups in a 2 x 4 factorial design (two feeding rates and four temperatures).

c. Fish were fed a mixture of 90% trout feed (70:30, floating: sinking) and 10% rabbit feed. Floating trout feed and rabbit pellets were screened to the size of the no. 4 sinking trout feed crumbles.

Details of the methods are listed in the experimental protocol (Appendix 12).

3. RESULTS

a. Analysis of variance of final weights and percent weight gain did not detect differences ($p>0.05$) among main effects (feed and temperature) or interactions, despite the observation that at the 4% feeding rate fish lost 2.3% of body weight at 5°C and gained 8.8% at 15°C (Table 1).

b. Least squares estimates of error were 5.6 and 4.8, respectively, accounting for the inability to detect differences at the sampling intensity.

c. Overall, the percent bodyweight gains ranging from -0.8 to 6.7% illustrated the influence of temperature on weight gain.

d. As expected, at 15°C the growth of grass carp was comparable to those held at 20°C; however, between 10 and 15°C the growth of fish was reduced with both feeding rates.

e. Excluding the 5°C water test groups, grass carp fed 4% of body weight at the low test temperatures, gained more than those fed 8% of body weight per day (Table 1).

f. Although growth would not be great, the results of this pilot study suggested that grass carp released in southern Alberta dugouts containing cold water (8-15°C) would survive until the water warmed.

g. Based on this study, a feeding rate of 2-4 % bodyweight per day was successfully implemented to hold approximately 1000 grass carp for 8 weeks at 11°C in Centre facilities.

Table 1. Mean (±SD) weights and percent weight gain of grass carp fed two feeding rates at low water temperature.

Growth Parameter	Feeding Rate (% bd wt/day)	Water Temperature (°C)[a] 5	10	15	20
Initial weight	4	8.37 (0.57)	8.19 (0.64)	7.31 (0.65)	7.55 (0.66)
	8	7.90 (0.68)	8.27 (0.44)	8.00 (0.80)	7.62 (0.64)
	$\bar{x}$	8.13 (0.64)	8.22 (0.55)	7.65 (0.78)	7.58 (0.62)
Final weight	4	8.17 (0.91)[b]	8.45 (0.69)	7.95 (0.63)	8.14 (0.86)[c]
	8	7.91 (0.95)	8.49 (0.47)	8.41 (0.95)	8.04 (0.53)
	$\bar{x}$	8.01 (0.88)	8.47 (0.56)	8.21 (0.81)	8.09 (0.67)
% Weight gain	4	-2.4 (10.8)	3.3 (8.4)	8.8 (8.7)	7.8 (11.3)
	8	0.2 (12.0)	2.7 (5.7)	5.1 (11.9)	5.5 (6.9)
	$\bar{x}$	-0.8 (10.9)	3.0 (6.8)	6.8 (10.1)	6.7 (9.0)

[a]Treatment means are not different ($p>0.05$) ($N = 5$).
[b]$N = 3$ after 14 days.
[c]$N = 4$ after 14 days.

References

1. Shireman, J.V., and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

2. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

APPENDIX 7

EXPERIMENTAL PROTOCOL

ASSESSMENT OF COMMERCIAL FEEDSTUFFS FOR GROWTH OF GRASS CARP FRY REARED IN TANKS

2440-CD5-2/P1

I. ADMINISTRATIVE INFORMATION.

A. Program: Toxicology of Biocides (CD)

B. Project/Sub-Project: Maintenance and Evaluation of Triploid Grass Carp (Ctenopharyngodon idella) (CD5-2)

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Assessment of commercial feedstuffs for growth of grass carp fry reared in tanks.
2. Number: 2440-CD5-2/P1
3. Authors: J.D. Somers
4. Word Processing File I.D. 1210G
5. Dates Written and Revised: September 13, 1988, September 30, 1988
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: September 23, 1988
   b. Actual:
8. Ending Date.
   a. Anticipated: October 27, 1988
   b. Actual:
9. Duration: 14 days

D. Client Department, Contact Person and Date for Final Report.

1. Client Department: Agriculture
2. Contact Person: D. Lloyd
3. Date Final Report Due: March 31, 1989

E. Principal Investigator(s), Participants and Levels of Responsibility.

1. Principal Investigator: J.D. Somers
2. Participants: K. Smiley, B. Goski, G. Sgouromitis, J. Schneider, J. Moore, L.E. Lillie
3. Responsibility:
   a. Animal Care: K. Smiley
   b. Statistics: Z. Florence, J. Somers
   c. Q/A, Q/C: J.A. Miller
   d. Monitoring: J.D. Somers
   e. Writing Report(s): J.D. Somers

F. Location of Study: Aquatic Biology Laboratory, Room B167

G. Test Agent and Hazard: Commercial feedstuffs will be evaluated. Hazard is nil.

H. Animals and Husbandry.

1. Animals.
   a. Species: Grass carp (Ctenopharyngodon idella)
   b. Strain or breed: Not known
   c. Sex: Not known
   d. Body weight at start of test: ~1.0 g
   e. Age: 4 months
   f. Acclimation/Acclimatization: 4 months AEC facilities
   g. Number of animals: 45

2. Husbandry.
   a. Housing and Caging: 37-L aquaria
   b. Feed: Commercial trout feed, frozen brine shrimp, alfalfa pellets
   c. Water: Recycled dechlorinated municipal
   d. Animal care SOPs:
      i. Euthanasia of Fish: 2350-AJ4/PR/EUTH/1
      ii. Disposal of Fish: 2350-AJ4/PR/NEC/6
      iii. Receipt, Acclimation and Quarantine of Rainbow Trout: 2350-AJ4/AN/AQ/11
   e. Animal identification: Fish lots per treatment group assigned accession number

II. Background, Objectives and Experimental Design.

A. Background

Grass carp are cultured primarily in earthen ponds containing natural vegetation. Under intensive cage culture grass carp can grow to 10 g in eight weeks, and to 60 g in the next six weeks (Shireman and Smith, 1983). Rearing facility limitations and quarantine restrictions at the Centre will not yield these growth rates.

Since receipt at the Centre as 1.5 mg larvae, 4000+ grass carp have grown to weights ranging from 1-5 g. Proper feeding of grass carp is required for growth and to prevent the induction of nutrient deficiencies (NRC, 1977). Grass carp growth can be influenced by feed type and quality, feed form and manner of presentation, water temperature, age and size of fish, and

fish density (Stickney, 1986). Preliminary feeding trials are required to evaluate these effects on grass carp growth and maintenance in rearing tanks at the Centre. Information gathered will be incorporated into husbandry programs at the Centre as the grass carp grow. Proper husbandry will ensure that fish of a suitable size and quality are available for stocking in 1989.

B. Objective(s)

The objective of this pilot study is to evaluate the growth rate of grass carp fry fed commercially available feedstuffs. Subsequent protocols will provide further evaluation of feed on grass carp growth.

C. Experimental Design

1. Study Design

a. Limitations on fish housing restrict the trial to a pilot study.
b. Three combinations of trout feed, brine shrimp and alfalfa will be evaluated (Table 1) in this study.
c. Daily feed will be given at the rate of 10% grass carp body weight.
d. Water temperature will be 20 ± 0.5°C.

2. Assignment to Treatment Groups

a. Forty-five grass carp will be randomly selected from the fish maintenance tank.
b. Fifteen grass carp will be randomly assigned to each of three dietary treatment groups.
c. Treatment groups will be arranged in a completely randomized design.
d. Treatment groups will not be replicated.

3. Parameters

Growth rates of individual fish will be determined by measuring body weight and fork length at the start and termination of the trial. Response will be represented by means for each parameter per treatment group because individual fish cannot be marked. Any subsequent analyses will be at the discretion of the principal investigator.

III. Experimental Procedures

A. Detailed Description

1. Selection of Test Grass Carp

a. Fifteen grass carp will be randomly selected for each of three treatment groups.
b. Each fish selected will be anesthetized in 1400 mL of recycled dechlorinated water containing 0.9 g tricaine methanesulfonate (MS222)/L (SOP 2350-AJ4/PR/EUTH/1).
c. The fork length and wet weight of each fish will be recorded.
d. Fish will be allowed to recover in 1500 mL of recycled water at 20°C, and then placed in the test aquaria.

2. Test Chamber Maintenance

a. Test aquaria will contain 37-L of recycled water at 20°C.
b. Each aquarium will be aerated and contain an Aquaclear Power Filter, and an aquarium heater.
c. About 25% of the water in each aquarium will be replaced each day.
d. Exposed surfaces of each aquarium will be covered with black plastic to reduce excitability of the test fish.
e. Excess feed and feces will be siphoned from the tanks each day.
f. Feces and waste water will be chlorinated before disposal (SOP 2350-AJ4/AN/AQ/11).

3. Feeding Regimes

a. Commercially available size no. 2 trout fry feed (52% protein), frozen brine shrimp (5.02% protein, 90% moisture) and pelleted alfalfa will be used for feeding fish.
b. The components of each test ration will be fed separately.
c. Fish will be fed twice daily (AM and PM).
d. The following test rations will be fed (Table 1):
   i. 50% trout feed, 45% frozen brine shrimp, 5% alfalfa
   ii. 70% trout feed, 25% frozen brine shrimp, 5% alfalfa
   iii. 95% trout feed, 0% frozen brine shrimp, 5% alfalfa
e. Trout feed and alfalfa will be fed dry, while frozen brine shrimp will be fed wet.

f. Alfalfa pellets (17% protein) will be crushed gently and screened to the size of a no. 2 trout feed crumble.
g. Frozen brine shrimp will be thawed and mixed in a blender before feeding.
h. The trial duration will be 14 days.

B. Monitoring

1. Any dead fish will be removed, and their wet weights and fork lengths recorded.
2. Wet weights and fork lengths of test fish will be recorded after 14 days.
3. Any necropsy or histochemical examination will be at the discretion of the principal investigator.
4. The pH, dissolved oxygen, temperature and conductivity of water in each test aquarium will be measured at the start of the test and at 24-h intervals during the trial.

C. Termination

1. Fish surviving after 14 days will be killed (SOP 2350-AJ4/PR/EUTH/1) and incinerated (SOP 2350-AJ4/PR/NEC/6).

D. Assessment and Interpretation

1. This pilot study is exploratory and preliminary. The mean change in fish body weight among treatment groups will be analyzed by one-way ANOVA using initial mean weight as a covariate.

2. The change in fish body weight will provide an estimate of growth and proper feeding rates.

IV. References

1. National Research Council. 1977. Nutrient requirements of warmwater fishes. National Academy of Sciences, Washington, DC. 78 pp.

2. Shireman, J.V. and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

3. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

Table 1. Feeding regimes for evaluation of grass carp fry growth.

Treatment group	No. of fish	Feeding rate (% body wt.)	Feeding regime[a,b]
1	15	10	50% no. 2 trout fry feed 45% frozen brine shrimp 5% alfalfa pellets
2	15	10	70% no. 2 trout fry feed 25% frozen brine shrimp 5% alfalfa pellets
3	15	10	95% no. 2 trout fry feed 0% frozen brine shrimp 5% alfalfa pellets

[a] The g of total feed per day depends on the total g of fish body weight per treatment group.

[b] Water temperature of all treatment groups will be 20 ± 0.5°C.

APPENDIX 8

EXPERIMENTAL PROTOCOL

EFFECT OF WATER TEMPERATURE ON GROWTH OF TANK-REARED GRASS CARP

2440-CD5-2/P2

I. ADMINISTRATIVE INFORMATION.

A. Program: Toxicology of Biocides (CD)

B. Project/Sub-Project: Maintenance and Evaluation of Triploid Grass Carp (Ctenopharyngodon idella) (CD5-2)

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Effect of water temperature on growth of tank-reared grass carp
2. Number: 2440-CD5-2/P2
3. Authors: J.D. Somers
4. Word Processing File I.D. 1219G
5. Dates Written and Revised: September 21, 1988, September 23, 1988, September 30, 1988
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: September 29, 1988
   b. Actual:
8. Ending Date.
   a. Anticipated: November 14, 1988
   b. Actual:
9. Duration: 14 Days

D. Client Department, Contact Person and Date for Final Report.

1. Client Department: Agriculture
2. Contact Person: D. Lloyd
3. Date Final Report Due: March 31, 1989

E. Principal Investigator(s), Participants and Levels of Responsibility.

1. Principal Investigator: J.D. Somers
2. Participants: K. Smiley, B. Goski, G. Sgouromitis, J. Schneider, J. Moore, L.E. Lillie
3. Responsibility:
   a. Animal Care: K. Smiley
   b. Statistics: Z. Florence, J. Somers
   c. Q/A, Q/C: J.A. Miller
   d. Monitoring: J.D. Somers
   e. Writing Report(s): J.D. Somers

F. Location of Study: Aquatic Biology Laboratory, Room B167

G. Test Agent and Hazard: Commercial feedstuffs and water temperature will be tested. Hazard is nil.

H. Animals and Husbandry.

1. Animals.
   a. Species: Grass carp (Ctenopharyngodon idella)
   b. Strain or breed: Not known
   c. Sex: Not known
   d. Body weight at start of test: ~ 1.0 g
   e. Age: 4.5 months
   f. Acclimation/Acclimatization: >4 months in Aquatic Biology facilities
   g. Number of animals: 45

2. Husbandry.
   a. Housing and Caging: 37-L aquaria
   b. Feed: Commercial trout feed, frozen brine shrimp, alfalfa pellets
   c. Water: Recycled dechlorinated municipal
   d. Animal care SOPs:
      i. Euthanasia of Fish: 2350-AJ4/PR/EUTH/1
      ii. Disposal of Fish: 2350-AJ4/PR/NEC/6
      iii. Receipt, Acclimation and Quarantine of Rainbow Trout: 2350-AJ4/AN/AQ/11
   e. Animal identification: Fish lots per treatment group assigned accession number

II. Background, Objectives and Experimental Design.

A. Background

Grass carp are cultured primarily in earthen ponds containing natural vegetation (Stickney, 1986). Under intensive cage culture grass carp can grow to 10 g in eight weeks, and to 60 g in the next six weeks (Shireman and Smith, 1983). Rearing facility limitations and quarantine restrictions at the Centre will not yield these growth rates.

Since receipt at the Centre as ~7 mm larvae weighing ~1.5 mg, 4000+ grass carp have grown to weights ranging from 1-5 g. Proper feeding of grass carp is required for growth and to prevent the induction of nutrient deficiencies (NRC, 1977). Grass carp growth can be influenced by feed type and quality, feed form and manner of presentation, water temperature, age and size of fish, and fish density (Stickney, 1986).

Preliminary feeding trials are required to evaluate these effects on grass carp growth and maintenance in rearing tanks at the Centre. Information gathered will be incorporated into husbandry programs at the Centre as the grass carp grow. Proper husbandry will ensure that fish of a suitable size and quality are available for stocking in 1989.

Grass carp are presently being reared in maintenance tanks at 21 ± 1°C. Future rearing will use water maintained at 26 ± 1°C. Feed intake and growth of grass carp are directly related to water temperature. Improper feeding rates in relation to water temperature could effect growth and lead to nutrient deficiencies.

B. Objective(s)

The objective of this pilot study is to evaluate the effect of water temperature on growth of grass carp reared in tanks. Subsequent protocols will provide further evaluation of temperature on grass carp growth.

C. Experimental Design

1. Study Design

   a. Water temperatures tested will be 20 ± 1°C, 25 ± 1°C and 30 ± 1°C.
   b. Test fish will be fed at the rate of 10% body weight per day.
   c. Daily feed will consist of 70% no. 2 trout fry feed, 25% frozen brine shrimp and 5% alfalfa.
   d. Limitations on fish housing restrict the trial to a pilot study status.

2. Assignment to Treatment Groups

   a. Forty-five grass carp will be randomly selected from the fish maintenance tank.
   b. Fifteen grass carp will be randomly assigned to each treatment group.
   c. Treatment groups will be arranged in a completely randomized design.
   d. Treatments will not be replicated.

3. Parameters

   Growth rates of individual fish will be determined by measuring body weight and fork length at the start and termination of the trial. Response will be represented by

means for each parameter per treatment group because individual fish cannot be marked. Any subsequent analyses will be at the discretion of the principal investigator.

III. Experimental Procedures

A. Detailed Description

1. Selection of Test Grass Carp

a. Fifteen grass carp will be randomly selected for each of three treatment groups.
b. Each fish selected will be anesthetized in 1400 mL of recycled dechlorinated water containing 0.9 g tricaine methanesulfonate (MS222)/L (SOP 2350-AJ4/PR/EUTH/1).
c. The fork length and wet weight of each fish will be recorded.
d. Fish will be allowed to recover in 1500 mL of recycled water at 20°C, and then placed in the test aquaria.

2. Test Chamber Maintenance

a. Once fish are placed in the test aquaria, the water for the 25 and 30°C treatments will be slowly increased to the desired temperature. This will take about 5 hours.
b. Each test aquarium will contain 37 L of recycled water.
c. Each aquarium will be continuously aerated, heated by an aquarium heater and filtered by an Aquaclear Power Filter.
d. Exposed surfaces of each aquarium will be covered with black plastic to reduce excitability of the test fish.
e. Excess feed will be siphoned from the tanks each weekday, collected in aluminum trays, oven-dried at 60°C for 24 h and weighed.
f. Feces and waste water will be chlorinated before disposal (SOP 2350-AJ4/AN/AQ/11).

3. Feeding Regimes

a. Commercially available size no. 2 trout fry feed (52% protein), frozen brine shrimp (5.02% protein, 90% moisture) and pelleted alfalfa (17% protein) will be fed to all fish.
b. Each component of the diet will be fed separately.
c. Fish will be fed twice daily (AM and PM).
d. The feed will consist of:

i. 70% trout feed, 25% frozen brine shrimp, 5% alfalfa

e. Trout feed and alfalfa will be fed dry, while frozen brine shrimp will be fed wet.

f. Alfalfa pellets (17% protein) will be crushed gently and screened to the size of a no. 2 trout feed crumble.

g. Frozen brine shrimp will be thawed and mixed in a blender before feeding.

h. The trial duration will be 14 days.

B. Monitoring

1. Any dead fish will be removed and their wet weights and fork lengths recorded.
2. Wet weights and fork lengths of test fish will be recorded after 14 days.
3. Any necropsy or histochemical examination will be at the discretion of the principal investigator.
4. The pH, dissolved oxygen, temperature and conductivity of water in each test aquarium will be measured at the start of the test and at 24-h intervals for the duration of the trial.

C. Termination

1. Fish surviving after 14 days will be killed (SOP 2350-AJ4/PR/EUTH/1) and incinerated (SOP 2350-AJ4/PR/NEC/6).

D. Assessment and Interpretation

1. This pilot study is exploratory and preliminary. The mean change in fish body weight among treatment groups will be analyzed by one-way ANOVA using initial mean weight as a covariate.
2. The change in fish body weight will provide an estimate of growth and feeding rates over a range of temperatures.

IV. References

1. National Research Council. 1977. Nutrient requirements of warmwater fishes. National Academy of Sciences, Washington, DC. 78 pp.

2. Shireman, J.V. and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

3. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

Table 1. Water temperature of test chambers.

Treatment group	No. of fish	Water temperature of test chambers
1	15	20°C
2	15	25°C
3	15	30°C

[a] All fish will be fed a diet consisting of 70% trout feed, 25% frozen brine shrimp and 5% alfalfa at the rate of 10% of body weight per day.

APPENDIX 9

EXPERIMENTAL PROTOCOL

EFFECT OF DIFFERENT FEEDING RATES OF COMMERCIAL FEEDSTUFFS ON GROWTH OF TANK-REARED GRASS CARP

2440-CD5-2/P3

I. ADMINISTRATIVE INFORMATION.

A. Program: Toxicology of Biocides (CD)

B. Project/Sub-Project: Maintenance and Evaluation of Triploid Grass Carp (Ctenopharyngodon idella) (CD5-2)

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Effect of different feeding rates of commercial feedstuffs on growth of tank-reared grass carp.
2. Number: 2440-CD5-2/P3
3. Authors: J.D. Somers
4. Word Processing File I.D. 1220G
5. Dates Written and Revised: September 21, 1988, September 30, 1988, October 14, 1988
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: October 17, 1988
   b. Actual:
8. Ending Date.
   a. Anticipated: November 25, 1988
   b. Actual:
9. Duration: 14 days

D. Client Department, Contact Person and Date for Final Report.

1. Client Department: Alberta Agriculture
2. Contact Person: D. Lloyd
3. Date Final Report Due: March 31, 1989

E. Principal Investigator(s), Participants and Levels of Responsibility.

1. Principal Investigator: J.D. Somers
2. Participants: K. Smiley, B. Goski, G. Sgouromitis, J. Schneider, J. Moore, L.E. Lillie
3. Responsibility:
   a. Animal Care: K. Smiley
   b. Statistics: Z. Florence, J. Somers
   c. Q/A, Q/C: J.A. Miller
   d. Monitoring: J.D. Somers
   e. Writing Report(s): J.D. Somers

F. Location of Study: Aquatic Biology Laboratory, Room B167

G. Test Agent and Hazard: Commercial feedstuffs will be tested. Hazard is nil.

H. Animals and Husbandry.

1. Animals.
   a. Species: Grass carp (Ctenopharyngodon idella)
   b. Strain or breed: Not known
   c. Sex: Not known
   d. Body weight at start of test: ~2.0 g
   e. Age: 5.5 months
   f. Acclimation/Acclimatization: >5 months in Aquatic Biology facilities
   g. Number of animals: 90

2. Husbandry.
   a. Housing and Caging: 37-L aquarium
   b. Feed: Commercial trout feed, frozen brine shrimp, alfalfa pellets
   c. Water: Recycled dechlorinated municipal
   d. Animal care SOPs:
      i. Euthanasia of Fish: 2350-AJ4/PR/EUTH/1
      ii. Disposal of Fish: 2350-AJ4/PR/NEC/6
      iii. Receipt, Acclimation and Quarantine of Rainbow Trout: 2350-AJ4/AN/AQ/11
   e. Animal identification: Fish lots per treatment group assigned accession number

II. Background, Objectives and Experimental Design.

A. Background

Grass carp are cultured primarily in earthen ponds containing natural vegetation (Stickney, 1986). Under intensive cage culture grass carp can grow to 10 g in eight weeks, and to 60 g in the next six weeks (Shireman and Smith, 1983). Rearing facility limitations and quarantine restrictions at the Centre will not yield these growth rates.

Since receipt at the Centre as ~7 mm larvae weighing ~1.5 mg, 4000+ grass carp have grown to weights ranging from 1-5 g. Grass carp growth can be influenced by feed type and quality, feed form and manner of presentation, water temperature, age and size of fish, and fish density (Stickney,

1986). Preliminary feeding trials are required to evaluate these effects on grass carp growth and maintenance in rearing tanks at the Centre. Information gathered will be incorporated into husbandry programs at the Centre as the grass carp grow. Proper husbandry will ensure that fish of a suitable size and quality are available for stocking in 1989.

Shireman et al. (1977) indicated that intensive tank culture of grass carp in Florida is possible by feeding duckweed (Lemna spp.). Although 2.7 g grass carp gained 30-70 g in 88 days when fed duckweed at 7.2-7.4% of body weight for 88 days, the use of fresh aquatic vegetation for feed is not feasible because of the copious daily quantities needed (Shireman et al., 1977). Prepared pelleted fish diets can be fed grass carp, but daily intake must be sufficient to ensure a supply of essential amino acids, minerals and vitamins (Shireman et al., 1978). Underfeeding of fish can produce uneven growth and nutrient deficiencies (NRC, 1977; Hilton and Slinger, 1981; Stickney, 1986).

B. Objective

The objective of this pilot study is to evaluate the effect of different feeding rates of commercial feedstuffs on growth of grass carp reared in tanks at 20 and 25°C. Subsequent protocols will provide further evaluation of feeding rate on grass carp growth.

C. Experimental Design

1. Study Design

a. Test fish will be fed at the rate of 8, 10 and 12% bodyweight per day (Table 1).
b. Water temperatures will be 20 ± 1°C and 25 ± 1°C.
c. Daily feed will consist of 70% no. 2 and 3 trout fry feed, 25% frozen brine shrimp and 5% alfalfa.
d. The experimental design will be a 2 x 3 factorial with two temperatures and three feeding regimes.

2. Assignment to Treatment Groups

a. Ninety grass carp will be randomly selected from the fish maintenance tank.
b. Fifteen grass carp will be randomly assigned to each treatment group.
c. Treatment groups will be arranged in a completely randomized design.

d. Treatments will not be replicated.

3. Parameters

Growth rates of individual fish will be determined by measuring body weight and fork length at the start and termination of the trial. Response will be represented by means for each parameter per treatment group because individual fish cannot be marked. Any subsequent analyses will be at the discretion of the principal investigator.

III. Experimental Procedures

A. Detailed Description

1. Selection of Test Grass Carp

a. Fifteen grass carp will be randomly selected for each of six treatment groups.
b. Each fish selected will be anesthetized in 1400 mL of recycled dechlorinated water containing 0.9 g tricaine methanesulfonate (MS222)/L (SOP 2350-AJ4/PR/EUTH/1).
c. The fork length and wet weight of each fish will be recorded.
d. Fish will be allowed to recover in 1500 mL of recycled water at 20°C, and then placed in the test aquaria.

2. Test Chamber Maintenance

a. Once fish are placed in the test aquaria, the water temperature will be slowly increased to 25°C for this treatment group. This will take about 5 hours.
b. Each test aquarium will contain 37 L of recycled water.
c. Each aquarium will be continuously aerated, heated by an aquarium heater and filtered by an Aquaclear Power Filter.
d. Exposed surfaces of each aquarium will be covered with black plastic to reduce excitability of the test fish.
e. Excess feed will be siphoned from the tanks each weekday, collected in aluminum trays, oven-dried at 60°C for 24 h and weighed.
f. Feces and waste water will be chlorinated before disposal (SOP 2350-AJ4/AN/AQ/11).

3. Feeding Regimes

a. Commercially available size no. 2 and 3 trout fry feed (52% protein), frozen brine shrimp (5.02% protein,

90% moisture) and pelleted alfalfa (17% protein) will be fed to all fish.

b. Each component of the diet will be fed separately.
c. Fish will be fed at 0900 and 1400 h.
d. The feed will consist of 70% trout feed, 25% frozen brine shrimp and 5% alfalfa
e. Trout feed and alfalfa will be fed dry, while frozen brine shrimp will be fed wet.
f. Alfalfa pellets (17% protein) will be crushed gently and screened to the size of a no. 3 trout feed crumble.
g. Frozen brine shrimp will be thawed and mixed in a blender before feeding.
h. The trial duration will be 14 days.

B. Monitoring

1. Any dead fish will be removed and their wet weights and fork lengths recorded.
2. Wet weights and fork lengths of test fish will be recorded after 14 days.
3. Any necropsy or histochemical examination will be at the discretion of the principal investigator.
4. The pH, dissolved oxygen, temperature and conductivity of water in each test aquarium will be measured at the start of the test and at 24-h intervals for the duration of the trial.

C. Termination

1. Fish surviving after 14 days will be killed (SOP 2350-AJ4/PR/EUTH/1) and incinerated (SOP 2350-AJ4/PR/NEC/6).

D. Assessment and Interpretation

1. This pilot study is exploratory and preliminary. The mean change in fish body weight among treatment groups will be analyzed by ANOVA for a 2 x 3 factorial using initial mean weight as a covariable.
2. The change in body weight will provide an estimate of the proper feeding rate at fish maintenance temperatures of 20°C and 25°C.

IV. References

1. Hilton, J.W., and S.J. Slinger. 1981. Nutrition and feeding of rainbow trout. Can. Spec. Publ. No. 55 of Fisheries and Aquatic Sci., Fisheries and Oceans, Ottawa, ON. 15 pp.

2. National Research Council. 1977. Nutrient requirements of warmwater fishes. National Academy of Sciences, Washington, DC. 78 pp.

3. Shireman, J.V., D.E. Colle, and R.W. Rottman. 1977. Intensive culture of grass carp, Ctenopharyngodon idella, in circular tanks. J. Fish Biol. 11:267-272.

4. Shireman, J.V., D.E. Colle, and R.W. Rottman. 1978. Growth of grass carp fed natural and prepared diets under intensive culture. J. Fish Biol. 12:457-463.

5. Shireman, J.V., and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

6. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

Table 1. Daily feeding rates as percent grass carp body weight to study the effects on growth of tank-reared grass carp at 20°C and 25°C.

Treatment group	No. of fish	Water temperature (°C)	Daily feeding rate (% of fish body weight)[a]
1	15	20	8
2	15	20	10
3	15	20	12
4	15	25	8
5	15	25	10
6	15	25	12

[a]Fish will be fed a diet consisting of 70% trout feed, 25% frozen brine shrimp and 5% alfalfa.

APPENDIX 10

EXPERIMENTAL PROTOCOL

FEEDING COMMERCIAL RABBIT FEED AS A SOURCE OF PLANT MATTER FOR TANK-REARED GRASS CARP

2440-CD5-2/P4

I. ADMINISTRATIVE INFORMATION.

A. Program: Toxicology of Biocides (CD)

B. Project/Sub-Project: Maintenance and Evaluation of Triploid Grass Carp (Ctenopharyngodon idella) (CD5-2)

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Feeding commercial rabbit feed as a source of plant matter for tank-reared grass carp.
2. Number: 2440-CD5-2/P4
3. Authors: J.D. Somers
4. Word Processing File I.D. 1221G
5. Dates Written and Revised: September 21, 1988; October 4, 1988
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: January 10, 1989
   b. Actual:
8. Ending Date.
   a. Anticipated: February 14, 1989
   b. Actual:
9. Duration: 14 days

D. Client Department, Contact Person and Date for Final Report.

1. Client Department: Alberta Agriculture
2. Contact Person: D. Lloyd
3. Date Final Report Due: March 31, 1989

E. Principal Investigator(s), Participants and Levels of Responsibility.

1. Principal Investigator: J.D. Somers
2. Participants: K. Smiley, B. Goski, G. Sgouromitis, J. Schneider, J. Moore, L.E. Lillie
3. Responsibility:
   a. Animal Care: K. Smiley
   b. Statistics: Z. Florence, J. Somers
   c. Q/A, Q/C: J.A. Miller
   d. Monitoring: J.D. Somers
   e. Writing Report(s): J.D. Somers

F. Location of Study: Aquatic Biology Laboratory, Room B167

G. Test Agent and Hazard: Commercial feedstuffs will be tested. Hazard is nil.

H. Animals and Husbandry.

1. Animals.
   a. Species: Grass carp (Ctenopharyngodon idella)
   b. Strain or breed: Not known
   c. Sex: Not known
   d. Body weight at start of test: 2.5-3.0 g
   e. Age: 6 months
   f. Acclimation/Acclimatization: >5 months in Aquatic Biology facilities
   g. Number of animals: 60

2. Husbandry.
   a. Housing and Caging: 37-L aquarium
   b. Feed: Commercial trout feed, commercial rabbit feed
   c. Water: Recycled dechlorinated municipal
   d. Animal care SOPs:
      i. Euthanasia of Fish: 2350-AJ4/PR/EUTH/1
      ii. Disposal of Fish: 2350-AJ4/PR/NEC/6
      iii. Receipt, Acclimation and Quarantine of Rainbow Trout: 2350-AJ4/AN/AQ/11
   e. Animal identification: Fish lots per treatment group assigned accession number

II. Background, Objectives and Experimental Design.

A. Background

Grass carp are cultured primarily in earthen ponds containing natural vegetation (Stickney, 1986), but can also be maintained with prepared diets under intensive culture (Shireman et al., 1978). Grass carp growth is influenced by feed type and quality, feed form and manner of presentation, water temperature, age and size of fish, and fish density (Stickney, 1986). Preliminary feeding trails are needed to evaluate these effects on grass carp growth and maintenance in rearing tanks at the Centre to ensure that fish of a suitable size and quality are available for stocking in 1989.

Aquatic vegetation is impractical for laboratory feeding of grass carp because of its high moisture content and the copious quantities required (Shireman et al., 1978). Grass

carp can grow efficiently on pelleted feeds if animal matter is included in the diet (Shireman et al., 1977). Grass carp cannot digest fibre; however, plant matter aids in the digestion of animal feed (NRC, 1977a). Shireman et al. (1978) successfully grew grass carp by feeding catfish feed. Trout feed is mainly animal material because trout cannot digest vegetation (Hilton and Slinger, 1981). Alfalfa pellets have been used as a source of plant matter for grass carp at the Centre. These pellets rapidly disintegrate, however, when added to the water and are not consumed.

Pelleted rabbit feed (14% crude protein, 14% crude fibre) is about 40% alfalfa (NRC, 1977b) and the pellets are harder than pelleted alfalfa. In addition to serving as a source of plant matter for grass carp, rabbit feed is fortified with vitamins which will aid in preventing malformations.

B. Objective

The objective of this pilot study is to evaluate the use of pelleted rabbit feed as a source of plant matter for tank reared grass carp. Subsequent protocols will provide further evaluation of plant material for grass carp feeding.

C. Experimental Design

1. Study Design

   a. Test fish will be fed at the rate of 8 or 10% bodyweight depending on the results of an earlier trial.
   b. Water temperature will be 25 ± 1°C.
   c. The daily feed for 3 treatment groups will contain 5, 10 and 15% pelleted rabbit feed (Table 1).

2. Assignment to Treatment Groups

   a. Sixty grass carp weighting 2.5-3.0 g will be randomly selected from the fish maintenance tank.
   b. Ten grass carp will be randomly assigned to each replicate of a treatment group.
   c. Treatment groups will be arranged in a randomized complete block design.
   d. Treatment groups will be replicated twice.

3. Parameters

Growth rates of individual fish will be determined by measuring body weight and fork length at the start and termination of the trial. Response will be represented by means for each parameter because individual fish cannot be marked. Any subsequent analysis will be at the discretion of the principal investigator.

III. Experimental Procedures

A. Detailed Description

1. Selection of Test Grass Carp

a. Ten grass carp weighing 2.5-3.0 g will be selected for each replicate of 3 treatment groups.
b. Each fish selected will be anesthetized in 1400 mL of recycled dechlorinated water containing 0.9 g tricaine methanesulfonate (MS222)/L (SOP 2350-AJ4/PR/EUTH/1).
c. The fork length and wet weight of each fish will be recorded.
d. Fish will be allowed to recover in 1500 mL of recycled water at 25°C, and then placed in the test aquaria.

2. Test Chamber Maintenance

a. Fish will be placed in the test aquaria at 25°C.
b. Each test aquarium will contain 37 L of recycled water.
c. Each aquarium will be continuously aerated, heated by an aquarium heater and filtered by an Aquaclear Power Filter.
d. Exposed surfaces of each aquarium will be covered with black plastic to reduce excitability of the test fish.
e. Excess feed will be siphoned from the tanks each weekday, collected in aluminum trays, oven-dried at 60°C for 24 h and weighed.
f. Feces and waste water will be chlorinated before disposal (SOP 2350-AJ4/AN/AQ/11).

3. Feeding Regimes

a. Commercially available size no. 3 trout fry feed (52% protein) and pelleted rabbit feed (14% protein) will be fed to all fish.
b. Each component of the daily feedings will be fed separately.

c. Fish will be fed twice daily (0900 and 1400).
d. The feed will consist of (Table 1):
   i. 95% trout feed, 5% rabbit feed
   ii. 90% trout feed, 10% rabbit feed
   iii. 85% trout feed, 15% rabbit feed
e. Rabbit feed pellets will be crushed gently and screened to the size of a no. 3 trout feed crumble.

B. Monitoring

1. Any dead fish will be removed and their wet weights and fork lengths recorded.
2. Wet weights and fork lengths of test fish will be recorded after 14 days.
3. Any necropsy or histochemical examination will be at the discretion of the principal investigator.
4. The pH, dissolved oxygen, temperature and conductivity of water in each test aquarium will be measured at the start of the test and at 24-h intervals for the duration of the trial.

C. Termination

1. Fish surviving after 14 days will be killed (SOP 2350-AJ4/PR/EUTH/1) and incinerated (SOP 2350-AJ4/PR/NEC/6).

D. Assessment and Interpretation

1. This pilot study is exploratory and preliminary. The mean change in fish body weight among treatment groups will be analyzed by ANOVA using initial mean weight as a covariate.
2. This study will provide an evaluation of rabbit feed as a source of plant material for grass carp.

IV. References

1. Hilton, J.W., and S.J. Slinger. 1981. Nutrition and feeding of rainbow trout. Can. Spec. Publ. No. 55. Fisheries and Aquatic Sci., Fisheries and Oceans Canada, Ottawa, ON. 15 pp.

2. National Research Council. 1977a. Nutrient requirements of warmwater fishes. National Academy of Sciences, Washington, DC. 78 pp.

3. National Research Council. 1977b. Nutrient requirements of rabbits. National Academy of Sciences, Washington, DC. 30 pp.

4. Shireman, J.V., D.E. Colle, and R.W. Rottman. 1977. Intensive culture of grass carp, Ctenopharyngodon idella, in circular tanks. J. Fish Biol. 11:267-272.

5. Shireman, J.V., D.E. Colle, and R.W. Rottman. 1978. Growth of grass carp fed natural and prepared diets under intensive culture. J. Fish Biol. 12:457-463.

6. Shireman, J.V., and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

7. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

Table 1. Proportions of pelleted rabbit feed and trout feed to be used in grass carp feeding trials to evaluate rabbit feed as a source of plant matter.

Treatment group[a]	No. of fish	Daily feeding rate (% of fish body weight)[b]
1	20	95:5
2	20	90:10
3	20	85:15

[a]Each treatment group will have 2 replicates containing 10 fish.
[b]Daily feed will be given at 8 or 10% of fish body weight depending on results of an earlier trial.

APPENDIX 11

EXPERIMENTAL PROTOCOL

EFFECT OF FEED PARTICLE SIZE AND PRESENTATION OF COMMERCIAL FEEDSTUFFS ON GROWTH OF TANK-REARED GRASS CARP

2440-CD5-2/P5

I. ADMINISTRATIVE INFORMATION.

A. Program: Toxicology of Biocides (CD)

B. Project/Sub-Project: Maintenance and Evaluation of Triploid Grass Carp (Ctenopharyngodon idella) (CD5-2).

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Effect of feed particle size and presentation of commercial feedstuffs on growth of tank-reared grass carp.
2. Number: 2440-CD5-2/P5
3. Authors: J.D. Somers
4. Word Processing File I.D. 1250G
5. Dates Written and Revised: November 17, 1988/November 30, 1988.
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: December 6, 1988
   b. Actual:
8. Ending Date.
   a. Anticipated: January 13, 1989
   b. Actual:
9. Duration: 14 days

D. Client Department, Contact Person and Date for Final Report.

1. Client Department: Alberta Agriculture
2. Contact Person: D. Lloyd
3. Date Final Report Due: March 31, 1989

E. Principal Investigator(s), Participants and Levels of Responsibility.

1. Principal Investigator: J.D. Somers
2. Participants: K. Smiley, B. Goski, G. Sgouromitis, J. Schneider, J. Moore, L.E. Lillie
3. Responsibility:
   a. Animal Care: K. Smiley
   b. Statistics: Z. Florence, J. Somers
   c. Q/A, Q/C: J.A. Miller
   d. Monitoring: J.D. Somers
   e. Writing Report(s): J.D. Somers

F. Location of Study: Aquatic Biology Laboratory, Room B167

G. Test Agent and Hazard: Commercial feedstuffs will be tested. Hazard is nil.

H. Animals and Husbandry.

1. Animals.
   a. Species: Grass carp (Ctenopharyngodon idella)
   b. Strain or breed: Not known
   c. Sex: Not known
   d. Body weight at start of test: ~3.5 g and ~6.5 g
   e. Age: 6.5 months
   f. Acclimation/Acclimatization: >5 months in Aquatic Biology facilities
   g. Number of animals: 42

2. Husbandry.
   a. Housing and Caging: 37-L aquarium
   b. Feed: Commercial trout feed, pelleted rabbit feed
   c. Water: Recycled dechlorinated municipal
   d. Animal care SOPs:
      i. Euthanasia of Fish: 2350-AJ4/PR/EUTH/1,
      ii. Disposal of Fish: 2350-AJ4/PR/NEC/6
      iii. Receipt, Acclimation and Quarantine of Rainbow Trout: 2350-AJ4/AN/AQ/11
   e. Animal identification: fish lots per treatment group assigned accession number

II. Background, Objectives and Experimental Design.

A. Background.

Grass carp are cultured primarily in earthen ponds containing natural vegetation (Stickney, 1986). Under intensive cage culture grass carp can grow to 10 g in eight weeks, and to 60 g in the next six weeks (Shireman and Smith, 1983). Rearing facility limitations and quarantine restrictions at the Centre will not yield these growth rates.

Grass carp growth can be influenced by feed type and quality, feed form and manner of presentation, water temperature, age and size of fish, and fish density (Stickney, 1986). Preliminary feeding trials are required to evaluate these effects on grass carp growth and maintenance in rearing tanks at the Centre. Information gathered will be incorporated into

husbandry programs at the Centre as the grass carp grow. Proper husbandry will ensure that fish of a suitable size and quality are available for stocking in 1989.

Prepared pelleted fish diets can be fed to grass carp, but daily intake must be sufficient to ensure a supply of essential amino acids, minerals and vitamins (Shireman et al., 1977; 1978). Underfeeding of fish producing uneven growth and nutrient deficiencies (NRC, 1977; Hilton and Slinger, 1981; Stickney, 1986) can result because an improper feed particle size is fed. The particle size of feed to be fed is directly related to fish size. In addition, grass carp prefer feeding on matter suspended in the water, rather than bottom feeding (Shireman and Smith, 1983).

B. Objectives.

The objective of this pilot study is to evaluate the effect of feed size and manner of presentation of commercial feedstuffs on growth of grass carp reared in tanks at 25°C. Subsequent protocols will provide further evaluation of feeding regimes on grass carp growth.

C. Experimental Design.

1. Study Design:

   a. Test fish will be fed at the rate of 8 and 10.4 % bodyweight per day (Table 1).
   b. Water temperature will be 25 ± 1°C.
   c. Daily feed will consist of 90% no. 3 or 4 trout fry feed and 10% rabbit feed.
   d. The experimental design will be a 2 x 3 factorial with two sizes of fish and three feeding regimes.
   e. The number of fish per treatment group will be altered to equalize fish density (g/L).

2. Assignment to treatment groups:

   a. Forty-two grass carp will be randomly selected from the fish maintenance tank.
   b. Nine grass carp (~3.5 g) will be randomly assigned to each of three treatment groups and five grass carp (~6.5 g) will be randomly assigned to each of three treatment groups.
   c. Treatment groups will be arranged in a completely randomized design.

d. Treatments will not be replicated.

3. Parameters:

Growth rates of individual fish will be determined by measuring body weight and fork length at the start and termination of the trial. Response will be represented by means for each parameter per treatment group because individual fish cannot be marked. Any subsequent analyses will be at the discretion of the principal investigator.

III. Experimental Procedures.

A. Detailed Description.

1. Selection of Test Grass Carp

a. Nine grass carp weighing 3.3-3.8 g will be randomly selected for each of 3 treatment groups and 5 grass carp weighing 6.3-6.8 g will be randomly selected for each of three treatment groups.
b. Each fish selected will be anesthetized in 1400 mL of recycled dechlorinated water containing 0.9 g tricaine methanesulfonate (MS222)/L (SOP 2350-AJ4/PR/EUTH/1).
c. The fork length and wet weight of each fish will be recorded.
d. Fish will be allowed to recover in 1500 mL of recycled water at 25°C, and then placed in the test aquaria.

2. Test Chamber Maintenance

a. Each test aquarium will contain 37 L of recycled water at 25 ± 1°C.
b. Each aquarium will be continuously aerated, heated by an aquarium heater and filtered by an Aquaclear Power Filter.
c. Exposed surfaces of each aquarium will be covered with black plastic to reduce excitability of the test fish.
d. Excess feed will be siphoned from the tanks 1 h after feeding each weekday, collected in aluminum trays, oven-dried at 60°C for 24 h and weighed.
e. Feces and waste water will be chlorinated before disposal (SOP 2350-AJ4/AN/AQ/11).

3. Feeding Regimes

a. Commercially available trout feed and pelleted rabbit feed will be fed to each weight class of fish as follows:

i. 90% no. 3 sinking trout feed (52% crude protein (CP); Marten Feed Mills); 10% rabbit feed (16% CP; United Feeds).
ii. 90% no. 4 sinking trout feed (40% CP; Marten Feed Mills); 10% rabbit feed (16% CP; United Feeds).
iii. 90% no. 4 floating trout feed (40% CP; Marten Feed Mills; 10% rabbit feed (16% CP; United Feeds).

b. No. 3 sinking fish feed (52% CP) will be fed at the rate of 8% bodyweight per day, while no. 4 fish feed (40% CP) will be fed at the rate of 10.4% bodyweight per day.
c. Adjusting the feeding rate of no. 4 fish feed by 1.3 times ($\frac{5\frac{2}{3}}{4\frac{1}{3}}$) will provide equal amounts of CP per treatment group per day (4% of body weight).
d. Floating fish feed pellets are extruded and are larger than size no. 4 sinking, so the pellets will be crushed gently and screened to the size of a no. 4 sinking trout feed pellet.
e. Rabbit feed pellets will also be crushed and screened to the size of a no. 3 or 4 trout feed crumble.
f. Rabbit pellets sized to no. 3 or 4 will be fed to fish given no. 3 and 4 trout feed, respectively.
g. Each component of the daily diet will be weighed and fed separately.
h. Fish will be fed at 09:00 and 14:00 h.
i. The trial duration will be 14 days.

B. Monitoring.

1. Any dead fish will be removed and their wet weights and fork lengths recorded.
2. Wet weights and fork lengths of test fish will be recorded after 14 days.
3. Any necropsy or histochemical examination will be at the discretion of the principal investigator.
4. The pH, dissolved oxygen, temperature and conductivity of water in each test aquarium will be measured at the start of the test and at 24-h intervals for the duration of the trial.

C. Termination.

1. Fish surviving after 14 days will be killed (SOP 2350-AJ4/PR/EUTH/1) and incinerated (SOP 2350-AJ4/PR/NEC/6).

D. Assessment and Interpretation

1. This pilot study is exploratory and preliminary. The mean change in fish body weight among treatment groups will be analyzed by ANOVA for a 2 x 3 factorial using initial mean weight as a covariable.

2. The change in body weight will provide an estimate of the effect of fish feed size and presentation on growth of tank reared grass carp.

IV. References.

1. Hilton, J.W., and S.J. Slinger. 1981. Nutrition and feeding of rainbow trout. Can. Spec. Publ. No. 55 of Fisheries and Aquatic Sci., Fisheries and Oceans, Ottawa, ON. 15 pp.

2. National Research Council. 1977. Nutrient requirements of warmwater fishes. National Academy of Sciences, Washington, DC. 78 pp.

3. Shireman, J.V., D.E. Colle, and R.W. Rottman. 1977. Intensive culture of grass carp, Ctenopharyngodon idella, in circular tanks. J. Fish Biol. 11:267-272.

4. Shireman, J.V., D.E. Colle, and R.W. Rottman. 1978. Growth of grass carp fed natural and prepared diets under intensive culture. J. Fish Biol. 12:457-463.

5. Shireman, J.V., and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella, (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

6. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

Table 1. Factorial experimental design for evaluating the effects of feed size and presentation on growth of tank reared grass carp.

Fish weight group[a]	Fish feed group[b]		
	No. 3 sinking[c]	No. 4 sinking	No. 4 floating[d]
~3.5 g	N = 9	9	9
~6.5 g	N = 5	5	5

[a] Fish weight treatment groups for each feed treatment group will have the same fish density (~1 g fish/L water).
[b] Daily feed will consist of 90% trout feed and 10% pelleted rabbit feed.
[c] Fish in no. 3 sinking fish feed treatment groups will be fed at the rate of 8% body weight per day.
[d] Fish in No. 4 fish feed treatment groups will be fed at the rate of 10.4% body weight per day.

APPENDIX 12

EXPERIMENTAL PROTOCOL

GROWTH AND SURVIVAL OF GRASS CARP EXPOSED TO LOW WATER TEMPERATURES

2440-CD5-2/P6

I. ADMINISTRATIVE INFORMATION.

A. Program: Toxicology of Biocides (CD)

B. Project/Sub-Project: Maintenance and Evaluation of Triploid Grass Carp (Ctenopharyngodon idella) (CD5-2)

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Growth and survival of grass carp exposed to low water temperatures.
2. Number: 2440-CD5-2/P6
3. Authors: J.D. Somers
4. Word Processing File I.D. 1252G
5. Dates Written and Revised: November 16, 1988
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: January 4, 1989
   b. Actual:
8. Ending Date.
   a. Anticipated: January 20, 1989
   b. Actual:
9. Duration: 15 days

D. Client Department, Contact Person and Date for Final Report.

1. Client Department: Alberta Agriculture
2. Contact Person: D. Lloyd
3. Date Final Report Due: March 31, 1989

E. Principal Investigator(s), Participants and Levels of Responsibility.

1. Principal Investigator: J.D. Somers
2. Participants: K. Smiley, B. Goski, G. Sgouromitis, J. Schneider, J. Moore, L.E. Lillie
3. Responsibility:
   a. Animal Care: K. Smiley
   b. Statistics: Z. Florence, J. Somers
   c. Q/A, Q/C: J.A. Miller
   d. Monitoring: J.D. Somers
   e. Writing Report(s): J.D. Somers

F. Location of Study: Aquatic Biology Laboratory, Room B158

G. Test Agent and Hazard: Water temperature will be tested. Hazard is nil.

H. Animals and Husbandry.

1. Animals.
   a. Species: Grass carp (Ctenopharyngodon idella)
   b. Strain or breed: Not known
   c. Sex: Not known
   d. Body weight at start of test: ~ 8.0 g
   e. Age: 8.0 months
   f. Acclimation/Acclimatization: >7 months in Aquatic Biology facilities
   g. Number of animals: 40

2. Husbandry.
   a. Housing and Caging: 37-L aquarium
   b. Feed: Commercial trout feed, pelleted rabbit feed
   c. Water: Recycled dechlorinated municipal
   d. Animal care SOPs:
      i. Euthanasia of Fish: 2350-AJ4/PR/EUTH/1
      ii. Disposal of Fish: 2350-AJ4/PR/NEC/6
      iii. Receipt, Acclimation and Quarantine of Rainbow Trout: 2350-AJ4/AN/AQ/11
   e. Animal identification: Fish lots per treatment group assigned accession number

II. Background, Objectives and Experimental Design.

A. Background

Grass carp are cultured primarily in earthen ponds containing natural vegetation (Stickney, 1986). Under intensive cage culture grass carp can grow to 10 g in eight weeks, and to 60 g in the next six weeks (Shireman and Smith, 1983). Grass carp growth can be influenced by feed type and quality, feed form and manner of presentation, water temperature, age and size of fish, and fish density (Stickney, 1986).

Preliminary feeding trials are required to evaluate these effects on grass carp growth and maintenance in rearing tanks at the Centre. Information gathered will be incorporated into husbandry programs at the Centre as the grass carp grow. Proper husbandry will ensure that fish of a suitable size and

quality are available for stocking in 1989. Prepared pelleted fish diets can be fed grass carp, but daily intake must be sufficient to ensure a supply of essential amino acids, minerals and vitamins (Shireman et al., 1978). Underfeeding of fish can produce uneven growth and nutrient deficiencies (NRC, 1977; Hilton and Slinger, 1981; Stickney, 1986).

Although 25°C is the optional water temperature for grass carp growth (Stickney, 1986), the water in southern Alberta dugouts in April 1989, when fish are released, will be much colder. In addition, some grass carp reared at the Centre have attained body weights >20 g in seven months. The grass carp is tolerant to extremes in environmental conditions (Shireman and Smith, 1983); however, the tolerance of grass carp reared at the Centre to low water temperature needs evaluation before fish are released into cold water in southern Alberta. Also, the use of low water temperatures to retard grass carp growth without inducing nutrient deficiencies needs study.

B. Objective

The objective of this pilot study is to evaluate the tolerance of grass carp reared in tanks at 5, 10, 15 and 20°C. Subsequent protocols will provide further evaluation of low temperatures on grass carp growth and survival.

C. Experimental Design

1. Study Design

a. Test fish will be fed at the rate of 4 or 8% bodyweight per day (Table 1).
b. Water temperature will be 5 ± 1, 10 ± 1, 15 ± 1 and 20 ± 1°C.
c. Daily feed will consist of 90% no. 4 trout fry feed and 10% rabbit feed depending on results of an earlier trial.
d. The experimental design will be a 2 x 4 factorial with two feeding regimes and four temperatures.

2. Assignment to Treatment Groups

a. Forty grass carp weighing ~8 g will be randomly selected from the fish maintenance tank.
b. Five grass carp will be randomly assigned to each treatment group.
c. Treatment groups will be arranged in a completely randomized design.

d. Treatments will not be replicated.

3. Parameters

Growth rates of individual fish will be determined by measuring body weight and fork length at the start and termination of the trial. Response will be represented by means for each parameter per treatment group because individual fish cannot be marked. Any subsequent analyses will be at the discretion of the principal investigator.

III. Experimental Procedures

A. Detailed Description

1. Selection of Test Grass Carp

a. Five grass carp weighing ~8 g will be randomly selected for each of 8 treatment groups.
b. Each fish selected will be anesthetized in 1400 mL of recycled dechlorinated water containing 0.9 g tricaine methanesulfonate (MS222)/L (SOP 2350-AJ4/PR/EUTH/1).
c. The fork length and wet weight of each fish will be recorded.
d. Fish will be allowed to recover in 1500 mL of recycled water at 20°C, and then placed in the test aquaria.

2. Test Chamber Maintenance

a. The sides and bottom of test aquaria will be covered with 2.5 cm styrofoam insulation and placed in the Aquatic Biology cold room (B158) with the temperature at 5°C.
b. Aquaria will be filled with 20°C recycled water and aquaria heaters adjusted to 20, 15, 10 and 5°C as the water cools. Pre-setting of heaters will be completed the day before commencement of the trial.
c. Water used for pre-setting heater controls will be pumped out of each test aquarium and replaced with 37 L of recycled water at 20°C prior to transferring fish to the aquaria.
d. Once fish are placed in the test aquaria the heaters will be turned on as pre-set at 20, 15, 10 and 5°C.
e. The decline in water temperatures will be monitored, and by using partial water replacement, maintained at the rate of $\leq$3°C per hour, until the pre-set temperatures are achieved.
f. Fish will not be fed until 24 h after placement in the test aquaria.

g. Each aquarium will be continuously aerated, heated by an aquarium heater and filtered by an Aquaclear Power Filter.
h. Excess feed will be siphoned from the tanks each weekday, collected in aluminum trays, oven-dried at 60°C for 24 h and weighed.
i. Feces and waste water will be chlorinated before disposal (SOP 2350-AJ4/AN/AQ/11).

3. Feeding Regimes

a. Commercially available size no. 4 trout fry feed and pelleted rabbit feed (16% protein) will be fed to all fish.
b. Size no. 4 floating trout feed (40% protein, Martin Feed Mills) and pelleted rabbit feed (United Feeds) will crushed gently and screened to the size of a no. 4 sinking trout feed crumble.
c. Size no. 4 sinking trout feed crumbles (40% protein, Martin Feed Mills) will be fed as commercially available.
d. The feed will consist of 90% trout feed (70:30 floating: sinking) and 10% rabbit feed; unless the results of an earlier trial suggest less rabbit feed is necessary.
e. Each component of the daily diet will be weighed and hand-blended in a beaker before feeding.
f. Fish will be fed at 0900 and 1400 h each day.
g. The trial duration will be 15 days; one day for temperature adjustment followed by 14 days feeding.

B. Monitoring

1. Any dead fish will be removed and their wet weights and fork lengths recorded.
2. Wet weights and fork lengths of test fish will be recorded after 14 days.
3. Any necropsy or histochemical examination will be at the discretion of the principal investigator.
4. The pH, dissolved oxygen, temperature and conductivity of water in each test aquarium will be measured at the start of the test and at 24 h intervals for the duration of the trial.

C. Termination

1. Fish surviving after 15 days will be killed (SOP 2350-AJ4/PR/EUTH/1) and incinerated (SOP 2350-AJ4/PR/NEC/6).

D. Assessment and Interpretation

1. This pilot study is exploratory and preliminary. The mean change in fish body weight among treatment groups will be analyzed by ANOVA for a 2 x 4 factorial using initial mean weight as a covariable.
2. The change in body weight will provide an estimate of the growth and survival of grass carp at low water temperatures.

IV. References

1. Shireman, J.V., and C.R. Smith. 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). Food and Agriculture Organization of the United Nations. FAO Synopsis No. 135, Rome.

2. Stickney, R.R. 1986. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, FL.

Table 1. Factorial design to study the growth and survival of grass carp raised at low water temperatures.*

Feeding rate (% body weight)**	Water temperature (°C)			
	5	10	15	20
4	n = 5	5	5	5
8	5	5	5	5

* Main effects, feeding rate and water temperature, will be analyzed by ANOVA for a 2 x 4 factorial in a randomized complete block design.

** Fish averaging ~ 8g will be fed size no. 4 sinking (40% protein) and floating (40% protein) trout feed, and rabbit pellets (16% protein) crushed and screened to the size of a no. 4 trout feed crumble.